Western Corn Rootworm (Coleoptera: Chrysomelidae) Beetle Emergence from Weedy Cry3Bb1 Rootworm-Resistant Transgenic Corn

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ABSTRACT Three greenhouse experiments were conducted to evaluate western corn rootworm, *Diabrotica virgifera virgifera* LeConte, beetle emergence from individual pots containing glyphosate-tolerant transgenic corn, *Zea mays* L., expressing the Cry3Bb1 endotoxin from the soil bacterium *Bacillus thuringiensis* Berliner (MON863), nontransgenic glyphosate-tolerant isoline corn, grassy weeds (giant foxtail, *Setaria faberi* R.A.W. Herrm; and large crabgrass, *Digitaria sanguinalis* (L.) Scop.), and combinations thereof infested with 40 neonate larvae. In the first two experiments, pots with corn and weed combinations were treated with glyphosate 5 d after larval infestation to kill the weeds. The third experiment was similar to the first two except that untreated corn–weed combinations were added. In all three experiments beetle emergence varied significantly. Beetle recovery generally did not vary between the nontransgenic, nontransgenic + weeds, and MON863 + weeds. Significantly more beetles were recovered from MON863 + weeds than MON863 alone or weeds alone. Beetle emergence from MON863 + weeds was likely enhanced by larvae that initially survived on weeds before application of glyphosate. Preliminary data indicated that fecundity was highest from beetles reared on nontransgenic isoline corn and fewer eggs were laid by beetles reared on MON863 alone. Egg viability was generally lowest from beetles reared on MON863. The implications of these results in relation to insect resistant management are discussed.

KEY WORDS MON863, glyphosate, grassy weeds, *Diabrotica virgifera virgifera*

WESTERN CORN ROOTWORM, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is one of the major pests of corn, *Zea mays* L., in the Corn Belt (north central United States). Because the damage by the corn rootworm complex has such a negative impact on corn production, several management tactics, such as soil-applied insecticides, crop rotation, and foliar sprays (to reduce beetle populations) are used to control these pests. However, insecticide resistance (Meinke et al. 1998, Wright et al. 2000), behavioral variants (Levine et al. 2002), and extended diapause (Krysan et al. 1986) limit the effectiveness of these tactics in many areas. Given these problems, new tactics are necessary to maintain a viable management program for corn rootworms. One such tactic, transgenic technology with resistance to corn rootworm larval feeding, is being researched by several seed companies (Monsanto Co., Pioneer HiBred Inc./Dow Agrosciences Inc., and Syngenta Crop Protection Inc.). YieldGard Rootworm, also known as MON863, was registered for commercial use on 25 February 2003 and is the only product currently commercially available. This transgenic corn expresses the Cry3Bb1 endotoxin from the soil bacterium *Bacillus thuringiensis* (Bt) Berliner, and is available for sale as a "stack" with tolerance to the herbicide glyphosate.

For a Bt crop to be registered for commercial release, the Environmental Protection Agency (EPA) requires an insect resistance management plan. Recently, our group demonstrated in greenhouse studies that western corn rootworm larvae develop to at least the second instar on most grasses examined (Clark and Hibbard 2004, Oyediran et al. 2004, Wilson and Hibbard 2004). MON863 is less efficacious for larvae beyond the first instar (EPA Scientific Advisory Panel 2002). A comprehensive resistance management plan for the western corn rootworm requires information on the role of grassy weeds as alternate hosts and larval movement between susceptible and resistant plants. Recently, Hibbard et al. (2003, 2004) demonstrated that western corn rootworm larvae can move at least three plants down a row and across corn rows. Hibbard et al. (2005) demonstrated that this movement is affected by Cry3Bb1 roots. Movement of larvae from susceptible to transgenic plants or vice versa could

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adversely affect resistance management (Mallet and Porter 1992, Davis and Onstad 2000). Alternatively, the interaction of grassy weeds, corn rootworm, and transgenic, rootworm-resistant corn could affect resistance management in a positive way if additional susceptible beetles are produced from within the transgenic field.

The objective of this experiment was to determine whether more western corn rootworm beetles were produced from MON863 in combination with grassy weeds (killed with glyphosate) than MON863 without grassy weeds.

Materials and Methods

Greenhouse Experiments. *Trial 1.* An experiment was conducted in which two grassy weeds—giant foxtail, *Setaria faberi* R.A.W. Herrm, and large crabgrass, *Digitaria sanquinalis* (L.) Scop.—glyphosate-tolerant MON863, and its glyphosate-tolerant isoline were planted in 19-liter pots containing 2:1 (vol:vol autoclaved) soil/peat-based growing medium mixture (Promix, Premier Horticulture LTEÉ, Quebéc, Canada). Large crabgrass and giant foxtail had previously been demonstrated to be larval hosts of the western corn rootworm (Clark and Hibbard 2004). Drainage openings in the pots were fitted with a fine (114 μm per opening) stainless steel mesh (TWP, Inc., Berkeley, CA) to prevent larval escape (Clark and Hibbard 2004). The following treatment combinations were evaluated: 1) MON863 alone, 2) isoline alone, 3) MON863 + giant foxtail + large crabgrass with weed removal, 4) isoline + giant foxtail + large crabgrass with weed removal, and 5) giant foxtail + large crabgrass. The experiment was a randomized complete block replicated 10 times where each pot served as a replicate. Fertilizer (Peters Professional 20-20-20, Spectrum Group, St. Louis, MO) was applied as necessary, and plants were watered twice weekly. One week after plant emergence, gene checks were conducted on all MON863 plants by using kits provided by Monsanto company to assay for expression of Cry3Bb1 protein (available from EnviroLogix, Portland, ME). One month after planting, pots were infested with 40 neonate larvae by gently transferring them with a moistened paintbrush into a 1-cm-deep hole at the soil surface in each pot. Due to larval availability, seven replicates were from a diapausing western corn rootworm colony at the USDA–ARS Northern Grain Insects Laboratory, whereas the remaining three replicates were from a nondiapausing colony maintained at the Plant Genetics Research Unit, USDA–ARS, Columbia, MO. All corn + weed treatments were sprayed with glyphosate in a 2% solution of Roundup WeatherMax (Monsanto Corporation) 4 to 5 d after infestation. At 25 d after infestation, pots were covered with screens to monitor adult emergence. A photoperiod of 14:10 (L:D) h was maintained with 1000-W sodium bulbs (GE Lighting, Cleveland, OH). A temperature recorder (Dickson SL 4350C7C, Dickson, Addision, IL) was used during the entire experiment, and temperatures remained relatively constant at 27 ± 2°C. Beetle emergence was monitored and recorded a minimum of twice weekly until no beetles had been collected for 3 wk.

Trial 2. The above-mentioned experiment was repeated and designated as trial 2. Treatment combinations and experimental designs were the same as described in trial 1, except that only nondiapausing larvae were infested in all replicates.

Trial 3. The same experiment was repeated, but two additional treatment combinations were added: 1) MON863 + weeds (no glyphosate spray) and 2) isoline + weeds (no glyphosate spray). The remaining experimental procedures were the same as in previous trials. Also in this trial, two experiments were conducted, one with the diapausing colony used for seven replicates of trial 1 and one with the nondiapausing colony used for the remaining three replicates of trial 1 and all of trial 2. Each experiment was replicated ten times.

Fecundity Studies. Development of reproductive potential using a laboratory-controlled environment can provide insight into species population dynamics that could be manifested under field conditions (Boetel and Fuller 1997). Several studies have been published regarding the reproductive biology and adult longevity of the western corn rootworm (Ball 1957, Branson and Johnson 1973, Hill 1975).

In these studies, males and females that emerged from the same treatment combinations were allowed to mate among themselves. The number of crosses made for each treatment was not equal due to the differences in the number of beetles recovered from the treatments. Beetles were reared following the methods of Boetel and Fuller (1997) with a few modifications. Each mating pair was placed in a 7.6 by 5.7 by 5.7-cm plastic box (Gary Plastic Packing Company, Bronx, NY). Beetles were maintained using a modification of the Boetel and Fuller (1997) technique and held at a photoperiod of 14:10 (L:D) h and 25°C. At the bottom of each plastic box was a 2-cm layer of moistened silty loam soil that served as an oviposition substrate. Before placement in boxes soil had been sieved through a 70-mesh sieve and autoclaved. Soil was moistened to near saturation and scarified to serve as an oviposition substrate. Beetles were fed dried diet moistened with honey (Jackson 1985) and supplied with water source for 4 wk, when adults were removed. For the diapausing beetles, the oviposition chambers containing the soil were placed into cold storage at 4°C 10 d after removal of the adults for a period of 180 d to ensure termination of egg diapause as suggested by Fisher et al. (1994). After 180 d, eggs were washed in cold water in a 60-mesh sieve to remove them from the soil and transferred to a moistened filter paper in a petri dish (14 by 2 cm) with square grids. All eggs recovered were counted under a microscope (M3Z, Wild Co., Heerbrugg, Switzerland). For nondiapausing beetles, eggs were removed from the soil once per week for 4 wk by washing as described above, but in water at room temperature. After washing and counting, the eggs were then placed in a petri dish (14 by 2 cm) taped

Table 1. Beetle emergence (mean ± SE) of combined, diapausing, and nondiapausing adults from Trial 1

Treatment	Total		
	Combined[a]	Diapausing[b]	Nondiapausing[c]
MON863	0.30 ± 0.21b	0.14 ± 0.14c	0.66 ± 0.66bc
Isoline	3.90 ± 0.95a	3.00 ± 1.15a	6.00 ± 1.00ab
MON863 + Weeds[d]	3.30 ± 1.42a	1.00 ± 0.58abc	8.67 ± 2.73a
Isoline + Weeds	4.80 ± 2.38a	1.71 ± 0.75ab	12.00 ± 6.60a
Weeds	0.60 ± 0.33b	0.71 ± 047b	0.33 ± 0.33c

Means followed by the same lowercase letter(s) in a column are not significantly different ($P = 0.05$). Although untransformed data are shown, analysis was performed using square root (x + 0.5) transformation.
[a] $n = 10$ when combining data from two strains.
[b] $n = 7$ diapausing western corn rootworms.
[c] $n = 3$ nondiapausing western corn rootworms.
[d] Weeds treated (removed) with glyphosate 5 d after infestation.

with Parafilm M (American Can Company, Greenwhich, CT) and placed in a growth chamber 25 ± 0.5°C. Eggs were checked two times weekly, and final percentage of hatch was determined.

Statistical Analysis. Adult emergence data were analyzed as a randomized complete block design using the PROC GLM of the statistical package of SAS (SAS Institute 2002), and the least significant difference (LSD) was calculated ($\alpha = 0.05$). Beetle emergence data were transformed because the normal probability plot of residuals indicated that data were not normally distributed. The square root (x + 0.5) transformation was used because it was the most effective in stabilizing the variance (Snedecor and Cochran 1989). Egg data were not analyzed because of the inadequate sample size.

Results

All the MON863 plants that were checked tested positive in the gene check test, and all the isoline plants checked were negative. When weeds were sprayed, symptoms of aboveground chlorosis to the giant foxtail and large crabgrass occurred 3 d after spraying, and plants looked mostly dead after 5 d.

Trial 1 Beetle Recovery. When data from diapausing and nondiapausing beetles were pooled, significantly more ($F = 5.03$; df = 4, 36; $P = 0.0025$) beetles emerged from MON863 + weeds sprayed with glyphosate than MON863 alone or weeds alone (Table 1). The greatest number of beetles was recovered from the isoline with weeds treatment, and the lowest number of beetles was recovered from MON863 alone. There was no significant difference among the isoline, isoline + weeds sprayed with glyphosate, and MON863 + weeds sprayed with glyphosate in total number of beetles recovered (Table 1). Similar results were obtained when beetle production from the diapausing ($F = 2.99$; df = 4, 24; $P = 0.0392$) and nondiapausing strains ($F = 5.33$; df = 4, 8; $P = 0.0216$) were analyzed separately (Table 1), although weeds seemed to have more of an effect on the nondiapausing replications during trial 1. The first beetle that emerged from the MON863, weed, and MON863 + weeds sprayed with glyphosate treatments was 9, 18, and 2 d later, respectively, than the first beetle that emerged from the isoline treatment.

Table 2. Western corn rootworm beetle emergence (mean ± SE) for trial 2

Treatment	No. beetles
MON863	1.60 ± 0.31b
Isoline	3.90 ± 1.01a
MON863 + weeds[a]	3.30 ± 0.93ab
Isoline + weeds[a]	4.10 ± 0.94a
Weeds	0.00 ± 0.00c

Means ($n = 10$) followed by the same lowercase letter(s) in a column are not significantly different ($P = 0.05$). Although untransformed data are shown, statistics were performed using square root (x + 0.5) transformation.
[a] Weeds were killed with glyphosate 5 d after infestation.

Trial 2 Beetle Recovery. The number of beetles recovered varied significantly between treatment combinations ($F = 8.85$; df = 4, 36; $P < 0.0001$), but no significance was found between the number of beetles emerged from MON863 + weeds sprayed with glyphosate and isoline or isoline plus weeds sprayed with glyphosate (Table 2). The highest number of beetles was recovered from the isoline + weeds and the lowest from MON863 alone. No beetle was recovered from weeds alone (Table 2). The first beetle that emerged from the MON863 treatment was 4 d later than the first beetle that emerged from the isoline treatment. There was no delay in emergences from the MON863 + weeds sprayed with glyphosate treatments.

Trial 3 Beetle Recovery. The total number of beetles recovered varied significantly between treatments for both the nondiapausing strain ($F = 6.40$; df = 6, 54; $P < 0.0001$) and for the diapausing strain ($F = 3.17$; df = 6, 54; $P = 0.0098$). The total number of beetles recovered from the nondiapausing strain did not vary significantly among isoline, MON863 + weeds sprayed with glyphosate, and isoline + weeds sprayed with glyphosate (Table 3). Similar data were found in the diapausing strain, but isoline + weeds sprayed with glyphosate had more adults for unknown reasons

Table 3. Western corn rootworm beetle emergence (mean ± SE) for trial 3

Treatment	No. beetles	
	Nondiapausing	Diapausing
MON863	0.30 ± 0.21c	0.00 ± 0.00c
Isoline	3.00 ± 0.73a	0.40 ± 0.22b
MON863 + weeds (glyphosate treated)	1.60 ± 0.37ab	0.30 ± 0.15b
Isoline + weeds (glyphosate treated)	2.00 ± 0.73ab	1.10 ± 0.45a
Weeds	0.10 ± 0.10c	0.10 ± 0.10c
MON863 + weeds (untreated)	0.60 ± 0.30d	0.10 ± 0.10c
Isoline + weeds (untreated)	1.10 ± 0.40b	1.10 ± 0.37a

Means ($n = 10$) followed by the same lowercase letter(s) in a column are not significantly different ($P = 0.05$). Although untransformed data are shown, the analysis was performed using square root (x + 0.5) transformation.

Table 4. Eggs (mean ± SE) laid per female with percentage viability

Treatment	Total no. eggs per female/% egg hatch							
	Nondiapausing						Diapausing	
	Trial 1	% hatch	Trial 2	% hatch	Trial 3a	% hatch	Trial 1	% hatch
MON863	180.0c (1)	70.0	244.3 ± 24.7 (3)[a]	76.4	220.1 (1)	70.00	95.0 ± 6.0 (2)	69.1
Isoline	390.1 ± 1.8 (3)	81.6	404.3 ± 13.1 (4)	80.1	411.6 ± 7.26 (4)	71.60	278.3 ± 15.0 (4)	71.7
MON863 + weeds[b]	295.8 ± 9.2 (2)	72.5	384.3 ± 14.4 (5)	75.4	373.3 ± 7.49 (5)	73.35	210.0 ± 20.2 (2)	70.5
Isoline + weeds[b]	368.3 ± 18.0 (2)	75.0	398.6 ± 8.2 (4)	81.3	408.0 (1)	80.00	275.0 ± 5.0 (2)	77.5
Weeds	n/a[c]	n/a	n/a	n/a	n/a	n/a	n/a	n/a

[a] Numbers in parentheses represent the number of mating pairs
[b] Weeds were killed with glyphosate 5 d after infestation.
[c] n/a, not available.

(Table 3). The first beetle that emerged from the MON863, weeds, and MON863 + weeds sprayed with glyphosate was 16, 26, and 7 d later, respectively, than the first beetle that emerged from the isoline treatment in the nondiapausing portion of this experiment. For diapausing portion of the experiment, the first beetle that emerged from weeds and MON863 + weed sprayed with glyphosate was 18 and 21 d later, respectively, than the first beetle that emerged from the isoline treatments.

Fecundity Studies. The mean number of eggs laid per female are presented without analysis because of the low number of beetles (Table 4). In each trial, beetles reared on isoline plants had higher oviposition rates than beetles from the MON863 (Table 4). More eggs were laid from MON863 plus weeds than from MON863 alone, but this was still fewer than the number of eggs laid by females recovered from isoline plants (Table 4). The viability of eggs ranged from 69.1 to 81.6% and was generally slightly lower when the adults had been reared as larvae on MON863 (Table 4).

Discussion

Results from the three trials are similar. Generally, the total number of beetles recovered did not vary significantly among the isoline, isoline planted with weeds, and MON863 with weeds, and more beetles were recovered from MON863 + weeds than MON863 alone or weeds alone. Beetle emergence also was delayed fewer days behind the isoline treatment in the MON863 + weeds treatment than the MON863 alone or weeds alone treatments. These results document the potential role of weeds in the survival of western corn rootworm larvae on MON863. Movement of larvae from susceptible to transgenic plants or vice versa could adversely affect resistance management in several ways (Mallet and Porter 1992, Davis and Onstad 2000). In the MON863 + weeds treatment (subsequently sprayed with glyphosate), some western corn rootworm neonates apparently started their development on the roots of the grassy weeds. However, when the grassy weeds were killed with glyphosate, the larvae apparently moved to and completed their development on MON863 roots. By the time the larvae moved from the dead grassy weeds to MON863, they were most likely large enough to overcome the Cry3Bb1 dose expressed in MON863 and able to use it as food for development. Evidence suggests that MON863 is only effective against the first instar (EPA Scientific Advisory Panel 2002). The low numbers of beetles recovered from MON863 alone also suggest that the product killed most of the first instars that fed on it. The level of Cry3Bb1 expressed in Bt corn roots has been measured at between 60.2 and 80.7 ppm at a V4 corn growth stage and between 40.5 and 49.3 ppm at a V9 corn growth stage (Vaughn et al. 2005). The concentration (LC_{50}) of Cry3Bb1 that killed half of the neonate western corn rootworm larvae in a diet bioassay was 50–75 ppm (EPA Scientific Advisory Panel 2002). Obviously, the levels of Cry3Bb1 found in Bt corn is not an extremely high-dose like previous Bt products, yet root protection in basically every field trial to date is at least nominally better than standard insecticides, often statistically, significantly so (EPA Scientific Advisory Panel 2002, Vaughn et al. 2005). Because the corn rootworm product does not fit the high-dose refuge paradigm of other Bt crops, other aspects of this system could be unique as well. It could be that partial development on grassy weeds is a good thing in terms of resistance management if more susceptible adults are produced within the transgenic field. This will not be known until progeny can be evaluated.

Although we did not evaluate progeny on MON863, we did collect oviposition and egg viability data. In each trial, the greatest number of eggs laid per female was recorded in females from isoline plants, and the lowest was from MON863. Egg viability was also slightly lower in the MON863 alone treatment. These findings are in agreement with findings of Boetel et al. (1998) who documented a reduction in the fecundity of western corn rootworm beetles that were exposed to insecticide treatments larvae compared with untreated beetles. Oviposition was generally higher in our study when adults were reared on MON863 + weeds than when reared on MON863 (Table 4). However, sample size was not adequate for statistical analysis.

Data from trials 1 and 3 were collected on both a diapausing and a nondiapausing strain of the western corn rootworm. Trial 2 consisted only of the nondiapausing strain. In trial 1, the three replications with the nondiapausing strain had much more of an effect, in terms of increased adult emergence in the presence of weeds, than did the diapausing strain. The nondiapausing strain has been in colony for well >100 generations (Hibbard et al. 1999). However, this colony was never selected for survival on grassy weeds, and there is no reason to expect that it would have better survival on alternate hosts than other western corn rootworm colonies. The reason the nondiapausing colony seemed to do better on combinations of alternate hosts and corn cannot be explained.

Of 60 grass species recently evaluated for western corn rootworm growth and development, larvae survived for at least 10 d on 57 species and grew to the second instar on 50 of the 60 grass species evaluated (Clark and Hibbard 2004, Oyediran et al. 2004, Wilson and Hibbard 2004). Although the current study only evaluated a mixture of giant foxtail and large crabgrass, our data may be applicable to most grassy weed species similar in host status.

Our greenhouse results imply that corn rootworm neonates established and fed on grassy weeds and moved to MON863 glyphosate-tolerant rootworm-resistant corn after herbicide application. This behavior increases the risk that significant damage could occur on MON863 and lead to increased beetle production. However, under field conditions, are more beetles likely to be produced from MON863 when weeds are present than when weeds are not present? Are the beetles that are produced from MON863 plus weeds going to be of a susceptible genotype (creating a refuge within the transgenic field)? These are questions that will need to be addressed for a complete understanding of this system.

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References Cited

Ball, H. J. 1957. On the biology and egg-laying habits of the western corn rootworm. J. Econ. Entomol. 50: 126–128.

Branson, T. F., and R. D. Johnson. 1973. Adult western corn rootworm: oviposition, fecundity, and longevity in the laboratory. J. Econ. Entomol. 66: 417–418.

Boetel, M. B., and W. Fuller. 1997. Seasonal emergence-time effects on adult longevity, fecundity, and egg viability of northern and western corn rootworms (Coleoptera: Chrysomelidae). Environ. Entomol. 26: 1208–1212.

Boetel, M. B., W. B. Fuller, L. D. Chandler, and D. G. Hovland. 1998. Fecundity and egg viability of northern and western corn rootworm (Coleoptera: Chrysomelidae) beetles surviving labeled and reduced soil insecticide applications. J. Econ. Entomol. 91: 274–279.

Clark, T. L., and B. E. Hibbard. 2004. Comparison of nonmaize hosts to support western corn rootworm (Coleoptera: Chrysomelidae) larval biology. Environ. Entomol. 33: 681–689.

Davis, P., and D. W. Onstad. 2000. Seed mixtures as resistance management strategy for European corn borers (Lepidoptera: Crambidae) infesting transgenic corn expressing Cry1ab protein. J. Econ. Entomol. 93: 937–984.

EPA, Scientific Advisory Panel 2002. Corn rootworm plant-incorporated protectant non-target insect and insect resistance management issues, Part B: insect resistance management issues. http;//www.epa.gove/scipoly/sap/2002/august/august2002final.pdf.

Fisher, J. R., J. J. Jackson, and A. C. Lew. 1994. Temperature and diapause development in the egg of *Diabrotica barberi* (Coleoptera: Chrysomelidae). Environ. Entomol. 23: 464–471.

Hibbard, B. E., B. D. Barry, L. L. Darrah, J. J. Jackson, L. D. Chandler, L. K. French, and J. A. Mihm. 1999. Controlled field infestations with western corn rootworm (Coleoptera: Chrysomelidae) eggs in Missouri: effects of egg strains, infestation dates, and infestation levels on corn root damage. J. Kans. Entomol. 72: 214–221.

Hibbard, B. E., D. P. Duran, M. R. Ellersieck, and M. M. Ellsbury. 2003. Post-establishment movement of western corn rootworm larvae (Coleoptera: Chrysomelidae) in Central Missouri corn. J. Econ. Entomol. 96: 599–608.

Hibbard, B. E., M. L. Higdon, D. P. Duran, Y. M. Schweikert, and M. R. Ellersieck. 2004. Role of egg density on establishment and plant-to-plant movement by western corn rootworm larvae (Coleoptera: Chrysomelidae). J. Econ. Entomol. 97: 871–882.

Hibbard, B. E., T. T. Vaughn, I. O. Oyediran, T. L. Clark, and M. R. Ellersieck. 2005. Effect of Cry3Bb1 expressing transgenic corn on plant-to-plant movement by western corn rootworm larvae (Coleoptera: Chrysomelidae). J. Econ. Entomol. 98: 1126–1138.

Hill, R. E. 1975. Mating, oviposition patterns, fecundity and longevity of the western corn rootworm. J. Econ. Entomol. 68: 311–315.

Jackson, J. J. 1985. *Diabrotica* spp., pp. 237–254. *In* P. Singh and R. F. Moore [eds.], Handbook of insect rearing, vol. 1. Elsevier Publishers, Amsterdam, The Netherlands.

Krysan, J. J., D. E., Foster, T. F. Branson, K. R. Ostlie, and W. S. Cranshaw. 1986. Two years before the hatch: rootworms adapt to crop rotation. Bull. Entomol. Soc. Am. 32: 250–258.

Levine, E., J. L. Spencer, S. A. Isard, D. W. Onstad, and M. E. Gray. 2002. Adaptation of the western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. Am. Entomol. 48: 94–107.

Mallet, J., and P. Porter. 1992. Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? Proc. Soc. Lond. B. 250: 165–169.

Meinke, L. J., B. D. Siegfried, R. J. Wright, and L. D. Chandler. 1998. Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. J. Econ. Enotmol. 91: 594–600.

Oyediran, I. O., B. E. Hibbard, and T. L. Clark. 2004. Prairie grasses as alternate hosts of the western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 33: 740–747.

SAS Institute. 2002. SAS/STAT user's guide. SAS Institute, Cary, NC.

Snedecor, G. W., and W. G. Cochran. 1989. Statistical methods, 8th ed. Iowa State University Press, Ames, IA.

Vaughn, T. T., T. Cavato, G, Brar, T. Coombe, T. DeGooyer, S. Ford, M. Groth, A. Howe, S. Johnson, K. Kolacz, et al. 2005. A method of controlling corn rootworm feeding using a *Bacillus thuringiensis* protein expressed in transgenic maize. Crop Sci. 45: 931–938.

Wilson, T. A., and B. E. Hibbard. 2004. Host suitability of nonmaize agroecosystem grasses for the western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 33: 1102–1108.

Wright, R. J., M. E. Scharf, L. J. Meinke, X. Zhou, B. D. Siegfried, and L. D. Chandler. 2000. Larval susceptibility of an insecticide-resistant western corn rootworm (Coleoptera: Chrysomelidae) population to soil insecticides: laboratory bioassays, assays of detoxification enzymes, and field performance. J. Econ. Entomol. 93: 7–13.

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